AD

TECHNICAL MANUSCRIPT 281

EFFECT OF AIR IONS ON SUBMICRON T1 BACTERIOPHAGE AEROSOLS

John W. Happ
J. Bruce Harstad
Lee M. Buchanan

FEBRUARY 1966

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 281

EFFECT OF AIR IONS ON SUBMICRON T1 BACTERIOPHAGE AEROSOLS

John W. Happ

J. Bruce Harstad

Lee M. Buchanan

Physical Defense Division
DIRECTORATE OF MEDICAL RESEARCH

Project 1C622401A072

February 1966

ACKNOWLEDGMENTS

The authors thank Dr. G. Briggs Phillips for advice and for making available his collection of literature on air ions, Melvin Filler for electron microscopy, Carl Michael for skilled technical assistance, and Dr. Francis M. Wadley for analysis of the experimental data.

ABSTRACT

The effect of a high concentration of ionized air molecules on sampling submicron T1 phage aerosols of a submicron particle size was evaluated by comparing the phage recoveries of all-glass impingers (AGI-4) and Type 6 filter papers. Sampler recoveries of all ionized aerosols were less than the recoveries of non-ionized control aerosols. These reductions in recovery were greater with positive ions than with negative ions or ions of mixed polarity. The AGI-4 allowed considerable slippage, which was not affected by the air ions. Type 6 filter paper recoveries were less than AGI-4 recoveries. The air ions did not appear to affect the aerosol particle size as determined by an electron microscope.

I. INTRODUCTION

Whitby[1] developed a sonic jet ionizer that produces a high concentration of ionized air molecules (air ions) and investigated their effect on inert aerosols. Whitby, Lundgren, and Peterson[2] found that aerosol particles produced by the atomization of dilute solutions of dyes carried a high electric charge that made the aerosol particles irregular in size and shape. Neutralization of the aerosol with a mixture of positive and negative air ions produced a more uniform aerosol and eliminated the migration that normally occurs with charged aerosols.

The action of air ions on bacteria suspended in small drops has been studied by Kreuger, Smith, and Go[3] who concluded that positive and negative air ions accelerated the rate of death of staphylococci, apparently by direct action on the cells and also by increasing the rate of evaporation.

Phillips, Harris, and Jones[4] found that a high concentration of air ions increased the exponential decay rates of aerosols of S. marcescens. The major part of the aerosol decay in the absence of air ions was due to biological decay; in the presence of air ions most of the increase in decay was attributed to physical decay. Negative and positive ions caused similar increases in physical decay. In addition to the increase in physical decay, negative ions increased biological decay but positive ions did not.

This paper reports on the effects of air ions of positive, negative, and mixed polarities on the sampling and particle size of submicron aerosols of T1 coliphage at a low relative humidity. The submicron aerosols were produced with a Dautrebande aerosol generator from concentrated aqueous suspensions of purified phage. The air ions were produced by the Whitby[1] sonic jet ionizer. The methods for phage purification and for generating, sampling, and particle sizing submicron phage aerosols were those used by Harstad.[5]

II. METHODS AND MATERIALS

A. AIR ION APPARATUS

A sonic jet ionizer (Fig. 1) developed by Whitby that is capable of producing a high concentration of air ions was used in these tests. The ionizer consists of a sharp needle located upstream from an orifice plate. A positive, negative or alterating current is applied between the needle and the orifice plate to create a slightly visible corona at the tip of the needle. Air ions are formed as a stream of air passes through the

corona. The ions are then forced through the plate orifice at sonic speeds, thus freeing the ions from the electric field around the corona. The particular specifications of the sonic jet ionizer are listed below:

Voltage	3500 volts
Orifice diameter	1.6 mm
Needle spacing	1.9 mm
Ionized gas	dried and filtered air
Air pressure	29 psig
Air flow	87 liters per minute
Ions	(+), (-), or a mixture of positive and negative ions
Ion concentration	10^9 ions per cubic cm

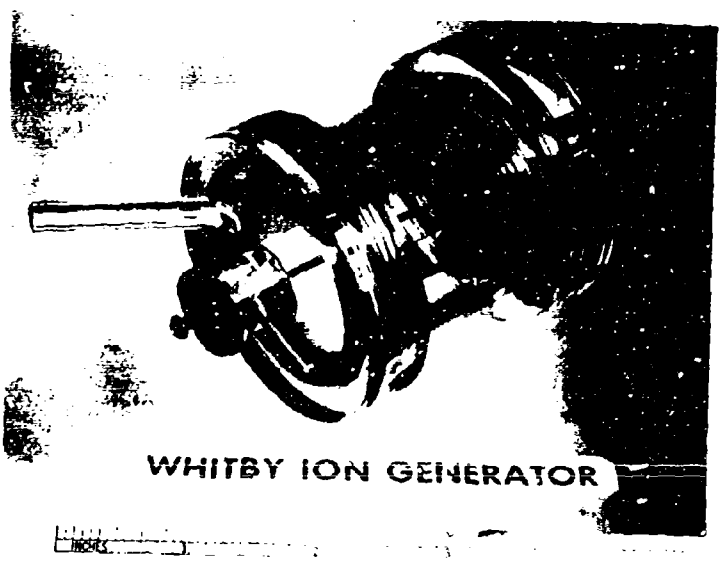

Figure 1. Whitby Sonic Jet Ionizer.

A Philco Ion Counter, Model ICG-6, was used to estimate the number of unit ion charges emitted by the sonic jet ionizer. Ozone was not detected by the stretched rubber test which is a simple approximate method used by Crabtree and Erickson[8] for measuring atmospheric ozone.

B. PHAGE AEROSOL GENERATION

The virus used in the aerosol tests was T1 bacteriophage, one of the viruses parasitic to Escherichia coli, strain B. Aqueous suspensions of T1 phage were prepared by concentrating and purifying large volumes of broth cultures by differential centrifugation and washing with distilled water. This thorough cleansing of the phage suspension to remove soluble and particulate contaminants was necessary to produce aerosols of minimal particle size. The concentration of the phage suspension used to fill the aerosol generator ranged from 0.5×10^{11} to 1.5×10^{11} phage particles per ml.

The Dautrebande $D_{30}1$ aerosol generator (J.H. Emerson Co., 22 College Park Ave., Cambridge, Mass.) was used to produce the submicron phage aerosols. The generator was operated at an air pressure of 17.5 psig, which resulted in an air flow through the generator of 18.4 liters per minute and a fluid atomization rate of 0.15 ml per minute. After leaving the generator, the aerosol passed into a 4-liter cylindrical glass chamber, then entered a 45-liter glass carboy where the aerosol was mixed with 87 liters of air containing the air ions. After leaving the carboy, the aerosol entered a circular sampling manifold to which the aerosol samplers were connected. The excess aerosol was bled off through a filter located upstream from the sampling manifold. The relative humidity was controlled by drying the air before it passed through the air ionizer and was monitored by wet and dry bulb thermometers attached to the carboy. Air supplies for aerosol generation and ion production were filtered, which assured clean particle-free air. The chamber was grounded to eliminate the residual charge that remained on the chamber walls after ionization. A schematic diagram of the aerosol chamber and apparatus is shown in Figure 2.

C. PHAGE AEROSOL SAMPLING

Two types of aerosol samplers, the all-glass impinger (AGI-4) (Ace Glass Co., Vineland, N.J.) and Chemical Corps Type 6 filter paper (Hollingsworth and Vose Co., East Walpole, Mass.) were used to determine the phage aerosol concentration in the presence or absence of added air ions. The all-glass impinger was filled with 22 ml of Bacto nutrient broth containing 0.10% Dow Corning Antifoam A (Dow Corning Corp., Midland, Michigan) and was operated at the maximum (approximately sonic) flow rate

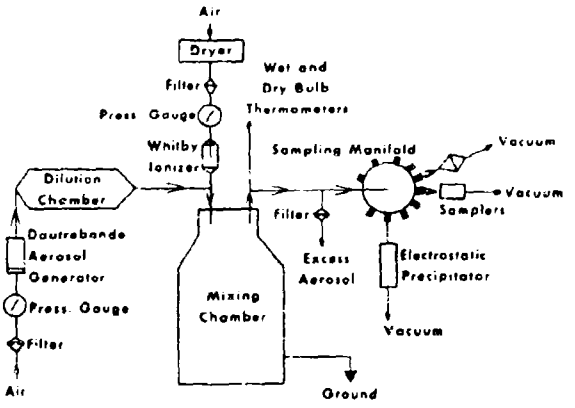

Figure 2. Diagram of Aerosol Apparatus.

of 12.5 liters per minute. The Chemical Corps Type 6 filter paper, an ultra-high-efficiency paper composed of Bolivian or African Blue asbestos, esparto grass, and kraft fibers was cut into discs 2.5 cm in diameter and sealed in in-line filter holders.[7] The sampling flow rate through the holders was 1.0 liter per minute. Immediately after sampling, the filter papers were placed in 100 ml of Bacto nutrient broth containing 0.1% Tween 20 (Atlas Powder Co., Wilmington, Delaware) and then shaken for 15 minutes on a mechanical shaker to disintegrate the paper.

Slippage of the phage aerosol through the all-glass impinger was determined by backing up the impinger with a filter holder containing Type 6 and Type 5 Chemical Corps filter papers. The Type 5 filter is a lower efficiency paper of cellulose and asbestos fibers with a backing of cotton scrim. It was used only to support the more fragile Type 6 paper and was assayed with the Type 6 paper.

To compare the size and shape of the phage aerosol particles before and after the addition of air ions, an electrostatic precipitator was used. The precipitator, which was connected to the sampling manifold, collected aerosol samples on an electron microscope specimen grid. The grid was then examined in an electron microscope, and photographs of the aerosol particles were taken.

Flow rates of sampler air were calibrated with a wet test meter. The all-glass impingers (AGI-4) were also calibrated for clearance of orifice to flask bottom and those with a clearance of 4 mm were selected for these tests.

Phage suspensions and aerosol samplers were assayed by making duplicate serial dilutions in Bacto nutrient broth and plating 1-ml samples of each dilution in triplicate, using an agar layer method described by Harstad.[5]

D. DESIGN OF EXPERIMENTS

The aerosol trials were designed to compare the effects of a high concentration of air ions of positive, negative, and mixed polarities on the sampling and particle size of submicron T1 phage aerosols. Each aerosol trial consisted of three consecutive tests conducted on a single day: a control test in which no air ions were added to the aerosol, a test in which air ions were added, and a final control test in which no air ions were added. The conditions of all tests were the same except for the addition of air ions. A series of 13 trials (days), consisting of 39 aerosol tests, was conducted. Five trials were conducted with negative ions and ions of mixed charge and three trials were conducted with positive ions. The schedule followed for each aerosol test consisted of filling the Dautrebande aerosol generator with 6 ml of the phage suspension and disseminating the phage aerosol continuously for 15 minutes, 10 minutes to establish equilibrium inside the aerosol mixing chamber followed by a 5-minute aerosol sampling period, and then 10 minutes to flush the aerosol chamber with clean, filtered, dry air. In the tests in which air ions were added, the Whitby sonic jet ionizer was turned on and off simultaneously with the Dautrebande aerosol generator. Except for the electrostatic precipitator, two samplers of each type were used and the samples pooled for phage assays.

III. RESULTS

A. PRELIMINARY EXPERIMENTS

In the first few aerosol tests conducted with air ions of mixed charge, the chamber was not grounded and sampler recoveries were noticeably higher during the second control test than during the first. To find the reasons for this phenomenon an electrometer was used to determine if there was a charge on the chamber walls prior to the first and second control tests. No charge was present prior to ionization and a positive charge did remain on the walls of the chamber after ionization was stopped and the aerosol chamber was flushed. When the chamber was grounded the residual charge was eliminated and sampler recoveries during the first and second control tests matched. Apparently, the positive charge remaining on the chamber walls after ionization caused a repulsion of the positively charged aerosol particles during the second control test, thereby permitting a greater number of organisms to be available for sampling. All data reported here were obtained when the chamber was grounded.

B. EFFECT OF ADDED AIR IONS ON SAMPLER RECOVERY

In Table 1 sampler recovery of ionized aerosols was compared with the recovery obtained for control (no ions added) aerosol as given by:

$$\text{Recovery} = \frac{\text{ionized aerosol recovery}}{\text{control aerosol recovery}}$$

AGI-4 and Type 6 filter paper recovery of all ionized aerosols was less than the recoveries of control aerosols. This loss in sampler recovery was more pronounced with positively charged aerosols than with negatively charged aerosols or aerosols of mixed charge. The recovery with negatively charged aerosols was somewhat less than that with aerosols of mixed charge, but the differences were not considered significant.

In Table 2, Type 6 filter paper recoveries were compared with AGI-4 recoveries as given by:

$$\text{Relative recovery} = \frac{\text{Type 6 filter paper}}{\text{AGI-4}}$$

Type 6 filter paper recoveries were less than AGI-4 recoveries for control aerosols and ionized aerosols. Air ions reduced recoveries with the Type 6 filter paper sampler to a greater extent than with the AGI-4 sampler (Table 1). Type 6 filter paper recovery was approximately 40% of AGI-4 recovery for each of the three ion treatments; this value was 63.5% for control aerosols.

TABLE 1. EFFECT OF AIR IONS ON THE SAMPLING OF SUBMICRON T1 PHAGE AEROSOLS

Sampler	Aerosol Condition	Number of Comparisons[a]	Mean Recovery,[b] %	Standard Error	95% Confidence Limits
AGI-4	Mixed ions added	10	48.3	4.31	38.6 - 58.0
	Negative ions added	10	43.6	5.79	30.5 - 56.7
	Positive ions added	6	11.4	2.25	5.6 - 17.2
Type 6 filter paper	Mixed ions added	10	34.4	2.76	28.2 - 40.6
	Negative ions added	10	30.0	4.36	20.1 - 39.9
	Positive ions added	6	7.6	1.79	3.0 - 12.2

a. Each trial yields two comparisons: $\frac{\text{ionized aerosol recovery}}{\text{first control aerosol recovery}}$ and $\frac{\text{ionized aerosol recovery}}{\text{second control aerosol recovery}}$

b. Mean aerosol temperature = 22 C Range = 20 to 25 C
Mean aerosol relative humidity = 29% Range = 24 to 32%

TABLE 2. COMPARISON OF TYPE 6 FILTER PAPER AND ALL-GLASS IMPINGERS (AGI-4) FOR THE RECOVERY OF IONIZED AND NON-IONIZED SUBMICRON T1 PHAGE AEROSOLS

Aerosol Condition	Number of Tests	Mean Relative Efficiency,[a] %	Standard Error	95% Confidence Limits
No ions added (control)	26[b]	63.5	2.94	57.4 - 69.6
Mixed ions added	5	43.2	5.57	27.7 - 58.6
Negative ions added	5	39.8	7.31	19.5 - 60.1
Positive ions added	3	40.8	0.47	38.8 - 42.8

a. Relative efficiency = $\frac{\text{Type 6 filter paper}}{\text{AGI-4}}$

b. Two control aerosol tests for each ionized aerosol test.

Table 3 shows the slippage of the phage aerosols through the AGI-4. Slippage was defined as:

$$\text{Slippage} = \frac{\text{Backup filter}}{\text{Backup filter + AGI-4}}$$

Slippage was slightly higher for ionized aerosols than for control aerosols but none of the differences was considered statistically significant.

C. EFFECT OF AIR IONS ON AEROSOL PARTICLE SIZE

Electron micrographs of phage aerosols showed no significant changes in size or shape between ionized and control aerosols. Figure 3 is an electron micrograph of a phage aerosol exposed to air ions of mixed charge.

TABLE 3. SLIPPAGE OF IONIZED AND NON-IONIZED SUBMICRON T1 PHAGE AEROSOLS THROUGH ALL-GLASS IMPINGERS (AGI 4)

Aerosol Condition	Number of Tests	Mean AGI-4 Slippage,[a] %	Standard Error	95% Confidence Limits
No ions added (control)	22[b]	20.8	1.05	18.6 - 23.0
Mixed ions added	4	23.2	0.51	21.6 - 24.8
Negative ions added	4	23.5	2.96	14.1 - 32.9
Positive ions added	3	22.0	1.89	13.9 - 30.1

a. Slippage = $\frac{\text{Backup filter}}{\text{Backup filter + AGI-4}}$

b. Two control aerosol tests for each ionized aerosol test.

Figure 3. Electron Micrograph of Particles from a T1 Phage Aerosol of Mixed Charge.

III. DISCUSSION

Previous studies on the sampling of submicron T1 phage aerosols in the absence of air ions have shown that biological loss such as death of the phage in the aerosol and killing of phage by the samplers is much larger than physical loss of the aerosol.[6] The present study revealed that air ions affected the stability of submicron T1 phage aerosols, resulting in a reduction of sampler recoveries. The reduced phage recoveries could be attributed to (i) aerosol death, which is a function of biological stability; (ii) physical aerosol loss from fallout or migration to the chamber walls; or (iii) killing of phage by the samplers, which is also a function of biological stability. The data do not reveal which of these factors was predominant. Slippage of the aerosol through the sampler was not a factor because ACI-4 slippage was not affected by the ion treatments and the previous study revealed that Type 6 filter paper is virtually a complete collector of submicron particles.

LITERATURE CITED

1. Whitby, K.T. 1961. Generator for producing high concentrations of small ions. Rev. Sci. Instrum. 32:1351-1355.

2. Whitby, K.T.; Lundgren, D.A.; Peterson, C.M. 1965. Homogeneous aerosol generators. Int. J. Air Water Pollut. 9:263-277.

3. Kreuger, A.P.; Smith, R.F.; Go, Ing Gan 1957. The action of air ions on bacteria: I. Protective and lethal effects on suspensions of staphylococci in droplets. J. Gen. Physiol. 41:359-381.

4. Phillips, G. Briggs; Harris, G.J.; Jones, M.W. 1964. Effect of air ions on bacterial aerosols. Int. J. Biometeor. 8:27-37.

5. Harstad, J. Bruce. 1965. Sampling submicron T1 bacteriophage aerosols. Appl. Microbiol. 13:899-908.

6. Crabtree, J.; Erickson, R.H. 1952. Atmospheric Ozone—A simple approximate method of measurement. India Rubber World 125:719-720.

7. Wolf, H.W.; Skally, P.; Hall, L.B.; Harris, M.M.; Decker, H.M.; Buchanan, L.M.; Dahlgren, C.M. 1959. Sampling microbiological aerosols. Public Health Monograph No. 60. U.S. Government Printing Office, Washington, D.C.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY *(Corporate author)*
U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland, 21701

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE
EFFECT OF AIR IONS ON SUBMICRON T1 BACTERIOPHAGE AEROSOLS

4 DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5 AUTHOR(S) *(Last name, first name, initial)*
Happ, John W.
Harstad, J. Bruce
Buchanan, Lee M.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
February 1966	18	7

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1C622401A072	Technical Manuscript 281
c.	9b OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10 AVAILABILITY/LIMITATION NOTICES
Qualified requestors may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701

13 ABSTRACT

The effect of a high concentration of ionized air molecules on sampling submicron T1 phage aerosols of a submicron particle size was evaluated by comparing the phage recoveries of all-glass impingers (AGI-4) and Type 6 filter papers. Sampler recoveries or all ionized aerosols were less than the recoveries of non-ionized control aerosols. These reductions in recovery were greater with positive ions than with negative ions or ions of mixed polarity. The AGI-4 allowed considerable slippage, which was not affected by the air ions. Type 6 filter paper recoveries were less than AGI-4 recoveries. The air ions did not appear to affect the aerosol particle size as determined by an electron microscope.

DD FORM 1 JAN 64 1473

Unclassified

Security Classification